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Jonathan D. Berman

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Walter Reed Army Institute of Research
Washington, DC 20307-5100

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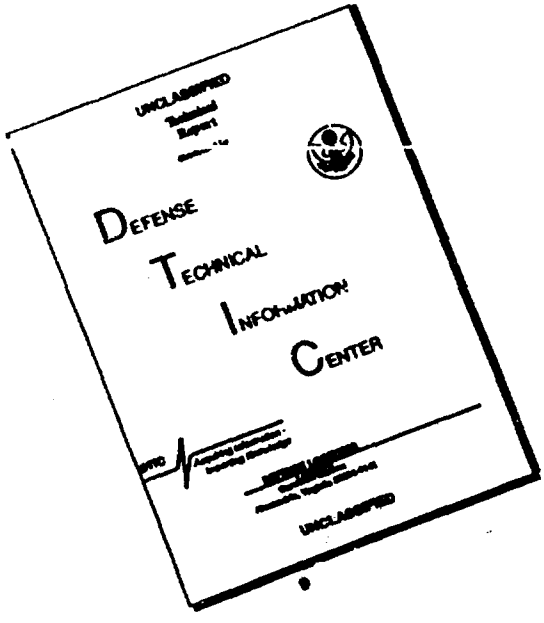

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Chapter 13

LEISHMANIASIS

Jonathan D. Berman

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I. CLINICAL LEISHMANIASIS

Leishmaniasis is caused by infection with protozoan parasites of the genus *Leishmania*. There are three major forms of clinical disease: cutaneous, mucosal, and visceral. Cutaneous disease typically presents as a papule on any part of the skin, which then progresses to a scabaceus ulcer with raised margins, and then heals with scarring. The first manifestation of disease occurred a median of 46 d after being bitten by the sandfly vactor in Guatemala, but the range of the latency period was wide (7 d to more than 12 months). There is also a wide range in the time over which the lesion will naturally heal. In the Guatemalan study, cutaneous disease due to *L. mexicana* that self-healed did so between 6 and 44 weeks after being brought to medical attention.[1] However, this data only pertains to lesions that were not chemotherapeutically treated because they appeared to be on the route to self-healing. The time for a natural cure for lesions that require chemotherapy is probably 1 to 2 years. Mucosal disease generally results from metastasis of organisms to the nasal-oral mucosa from previous cutaneous lesions. The time between healing of the cutaneous lesion and initiation of the nasal lesion has been reported as between 0.2 and 27 years.[2,3] The nasal mucosa is always first involved. If left untreated, the disease spreads over a period of months to years to involve the mucosa of the palate, uvula, pharynx, and larynx. Death, due to suffocation, is rare. Visceral leishmaniasis results from infection of the liver, spleen and, bone marrow with *Leishmania*. The symptoms of established disease are fever, weight loss, hepatosplenomegaly, and pancytopenia. If untreated, established disease is characteristically fatal due to intercurrent infections such as diarrhea and pneumonia. However, there are more patients with mild, subclinical disease than with established disease. In subclinically infected patients, modest symptoms may easily smolder for three years before resolution.[4]

II. *LEISHMANIA* LIFE CYCLE

Leishmaniasis is an arthopod-borne disease and is acquired via the bite of the female sandfly vector. The reservoirs of disease are animals such as desert rats *(L. major)*, sloths *(L. panamensis)*, horses *(L. braziliensis)*, rodents *(L. mexicana)*; and foxes and dogs *(L. donovani)*. In India, inadequately treated visceral infection may be succeeded by cutaneous lesions (post kala-azar dermal leishmaniasis), and man may be the reservoir for the spread of visceral *Leishmania* under these circumstances.

The female sandfly acquires the mammalian, rounded (3 × 4 μ) amastigote form of the organism as cutaneous tissue is ingested with a blood meal. Within the sandfly, amastigotes transform into the free-living, elongated (3 × 20 u) promastigote form. Recent work has shown that over a week's time, promastigotes present in the sandfly gut undergo antigenic change to become

metacyclic forms which, although morphologically indistinguishable from promastigotes, are more infective than promastigotes for mammals.[5] The shift from promastigotes to metacyclics involves the loss of a promastigote glycolipid which mediates peanut agglutinin reaction[6] and an alteration and doubling of the saccharide units in the major cell surface glycoconjugate lipophosphoglycan (LPG).[5,7-9] LPG is the major complement receptor on both promastigotes and metacyclics, but only promastigotes are lysed as a result of complement deposition. The larger size of LPG on the metacyclics may prevent deposited complement from inserting into the membrane and lysing the organism.[8,9] Injection of metacyclics into a mammal during the next blood meal completes the arthropod portion of the life cycle.

Free-living *Leishmania* are susceptible to mammalian bodily temperatures.[10,11] Either to avoid high temperature or for unidentified reasons, free-living forms rapidly (within hours) attach to professional phagocytic cells via the CR1 receptor for complement C3b that has been deposited on the parasite, and via the CR3 receptor that binds the major parasite surface glycoprotein, GP 63, and are phagocytized.[12] Lysosomes fuse with the phagosome containing the parasite, and the flagellated forms transform over a period of approximately 3 d into amastigotes located within the macrophage phagolysosome.

III. DEFENSE MECHANISMS OF *LEISHMANIA* AND HOST CELLS

The above discussion of the life cycle of Leishmania reveals the crucial pathophysiological fact that the mammalian form of the organism is only localized within the phagolysosomes of monocytes/macrophages. Amastigotes are not found free of host cells, and are not found in any cells other than monocytes/macrophages. Multiplication of amastigotes within the phagolysosomes of macrophages of the skin, mucosa, and visceral reticuloendothelial system results in cutaneous, mucosal, and visceral leishmaniasis, respectively.

Since phagocytized parasites multiply within the subcellular organelle designed to digest microorganisms, the parasites must have mechanisms that enable them to be resistant to microbicidal enzymes. LPG, in addition to mediating resistance to complement lysis, inhibits protein kinase C,[13] and the inhibition of protein kinase C may be the mechanism by which c-*fos* gene expression in the infected macrophage is inhibited.[14] The major surface glycoprotein of amastigotes, GP 63, is a zinc-containing proteinase with an acidic pH optimum appropriate to the intraphagolysosomal environment. Since liposomes can be protected from macrophage degradation by coating the liposomes with GP 63,[15] GP 63 may protect amastigotes from macrophage microbicidal mechanisms. Other modifications of macrophage metabolism that may protect the amastigote are parasite-induced alteration in mammalian cyclooxygenase and lipooxygenase pathways (such as the increase in

leukotriene-C4 in *L. donovani*-infected macrophages),[16] and diminished induction of the mRNA for the major histocompatibility class II protein in response to interferon-gamma (INF-γ) stimulation.[17]

On the other hand, cutaneous disease ultimately self-cures, and most visceral disease is subclinical and well controlled by the human host. Mechanisms by which human macrophages kill intracellular amastigotes are thought to involve reactive oxygen intermediates and reactive nitrogen intermediates. *In vitro, Leishmania* promastigotes are susceptible to H_2O_2 or to a xanthine oxidase/xanthine system which generates H_2O_2, O_2^-, and OH. Killing by xanthine oxidase/xanthine was 50% inhibited by catalase, which degrades H_2O_2, but was not inhibited by superoxide dismutase and other scavengers of O_2^- and OH.[18] These experiments suggested that promastigotes were susceptible to H_2O_2 but not to superoxide or to hydroxyl radical, and were consistant with the fact that promastigotes contain almost undetectible levels of catalase, but levels of superoxide dismutase comparable to that of another protozoan, *Toxoplasma gondii*.[18] Similar experiments were performed with amastigotes. Although amastigotes had three times the concentration of catalase and of superoxide dismutase than did promastigotes, the level of amastigote catalase was, nevertheless, less than 3% of the concentration in *T. gondii*.[19]

When ingested by human peripheral blood monocytes, promastigotes stimulated more H_2O_2 production than did amastigotes, and ~100% of promastigotes but no amastigotes were killed 72 h later. Lymphokine-stimulated monocytes had enhanced H_2O_2 production and an enhanced ability to kill amastigotes (50% at 72 h).[20] Although this work suggests that generation of H_2O_2 is the mechanism by which human mononuclear cells kill *Leishmania*, other lysosomal agents that were not measured might be increased in parallel with H_2O_2 and might participate in leishmaniacidal activity. In fact, macrophages from patients with chronic granulomatous diseases, which release merely 2% of the H_2O_2 released by normal macrophages, were activatable by lymphokines such that 30% of amastigotes were killed 48 h after ingestion.[20]

The lymphokine that activates human monocyte-derived macrophages to kill ingested *Leishmania* is INF-γ. Antibody to INF-γ abolished the ability of crude lymphokine to activate macrophages to kill *Leishmania*, and recombinant human INF-γ was as effective as crude lymphokine in activation of macrophages from both normal and chronic granulomatous disease patients.[21]

Experiments with murine macrophages suggest that reactive nitrogen intermediates constitute one oxygen-independent mechanism for the killing of amastigotes. Nitrogen from the guanidino group of L-arginine may be metabolized to the reactive nitrogen compounds nitrate, nitrite, and nitric oxide. The ability of INF-γ to activate mouse peritoneal macrophages to kill *L. major* amastigotes was suppressed by *n*-monomethyl-L-arginine, an inhibitor of the above pathway. Nitrite levels in the macrophage supernatant correlated with

antileishmanial activity.[22] In addition, injection of *n*-monomethyl-L-arginine into *L. major* lesions in mice exacerbated the lesions and resulted in a 10^4 increase in the number of parasites extractable from the lesions.[23] Tumor necrosis factor-a (TNF-a) has been implicated in the killing of intracellular *Leishmania* by activated mouse macrophages. Infection of mouse macrophages with amastigotes stimulates macrophage TNF-a production; INF-γ activation of infected macrophages results in an even higher level of TNF-a production. Since treatment of INF-γ activated, amastigote-infected macrophages with antibody to TNF-a abrogated macrophage antileishmanial activity, TNF-a generation by the mouse macrophage is required to kill the intracellular parasite.[24]

This neat picture by which INF-γ stimulates TNF-a production in amastigote-infected macrophages, resulting in reactive nitrogen intermediate killing of the parasite, has been recently called into question by work with infected human peripheral blood monocytes. When human monocytes were infected with *L. donovani* amastigotes, subsequent incubation with INF-γ reduced the number of parasites by 75% over 72 h. However, addition of the inhibitor of arginine metabolism (*n*-monomethyl-L-arginine) did not inhibit INF-γ induced antileishmanial activity nor INF-γ-induced anti-Toxoplasma activity, whereas *n*-monomethyl-L-arginine did inhibit by 50% anti-Toxoplasma activity in INF-γ-activated mouse macrophages. Murray concludes that the contribution of reactive nitrogen intermediates to antileishmanial activity is a species specific phenomenon that does not hold for human mononuclear cells.[25]

Although the precise biochemical mechanisms by which the host eliminates *Leishmania* from macrophages is unclear, it has long been recognized that cellular immune mechanisms, rather than humeral immune mechanisms, are protective against these diseases. For example, classic visceral leishmaniasis is easily diagnosed by antileishmanial antibodies at serum dilutions of 1:1000,[26] but there is no reaction to the *Leishmania* in the skin test[27] and the patient will die without chemotherapeutic intervention. Approximately 1 to 2 years after treatment of kala-azar,[27,28] the skin test converts to positive, and it is thought that cellular immunity maintains protection against symptomatic recurrence. In classic cutaneous leishmaniasis, which self-cures in months, there is a positive skin test[29] and the patients' peripheral blood monocytes secret INF-γ in response to specific antigenic stimulation.[30] In the rare form of cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, in which the patient is skin test negative and relapses after chemotherapy, conversion to skin test positivity is thought to correlate with the ability of chemotherapy to effect a cure.[31] The only exception to the rule that apparent healing of leishmanial lesions corresponds to the presence of cellular immunity is mucosal leishmaniasis. Here, *in vitro* lymphocyte blastogenesis and INF-γ secretion, in response to stimulation with the *Leishmania* antigen, is greater than that in cutaneous disease, yet the disease is progressive.[30]

IV. THERAPY OF THE LEISHMANIASES

A. INTRODUCTION

Some forms of antileishmanial therapy are based on the standard approach of administering a drug in the clinic that has been shown to have antileishmanial activity *in vitro*. Other modalities of treatment, however, are based at least partially on taking advantage of the precise intramacrophage niche occupied by the organism. The agents that are active against clinical leishmaniasis — pentavalent antimony, pentamidine, amphotericin B, ketoconazole, allopurinol, paromomycin, liposomes, INF-γ — will be reviewed on the basis of inherent antileishmanial activity, biochemical mechanisms of action, and specific concentration or interaction of the agent with infected macrophages. The structures of the antileishmanial agents are shown in Figure 1. The treatment regimens recommended by the author for the major *Leishmania* syndromes are shown in Table 1. The mechanisms of action of the antileishmanial agents are summarized in Table 2.

B. PENTAVALENT ANTIMONIALS

Treatment with pentavalent antimonials (Sb) has been the subject of prior review.[32] Trivalent antimonials such as tartar emetic were first used in 1912; less toxic pentavalent antimonials became available in the 1920s, and the present formulations of pentavalent antimonials that are still the first-line drugs for all clinical syndromes became available in the 1940s. The formulations presently in use are sodium stibogluconate (Pentostam), in which pentavalent antimony is reacted with gluconic acid to form an unknown number of compounds of unknown structure, and meglumine antimonate, in which pentavalent antimony is reacted with the sugar meglumine to form a similarly unknown set of products. Pentostam is marketed as a solution containing 100 mg Sb/ml and Glucantime is marketed as a solution containing 85 mg Sb/ml. Pentostam is used in traditionally English speaking countries and Glucantime is used in traditionally non-English speaking countries. No systematic comparison of the agents has been performed, and Sb in the two formulations is assumed to be equivalent with respect to efficacy and toxicity.

Very recent recommendations are that Sb be used at a daily dose of 20 mg Sb/kg. Treatment duration is 20 d for cutaneous disease, and 28 d for visceral and mucosal disease.[33] Although these recommendations constitute easy-to-remember blanket instructions, there are probably exceptions in which other regimens would be preferable. For cutaneous disease, a treatment duration of 20 d was chosen because >90% of patients with disease acquired in Central America healed with such a regimen. Shorter treatment durations have not been tested, and it is likely that disease due to some species of *Leishmania* will only require 10 or 15 d of treatment. The 28-d treatment regimen for visceral disease will generally give rise to clinical cure in >90% of patients, but in India 40 d of therapy (97% cure) may be advantageous

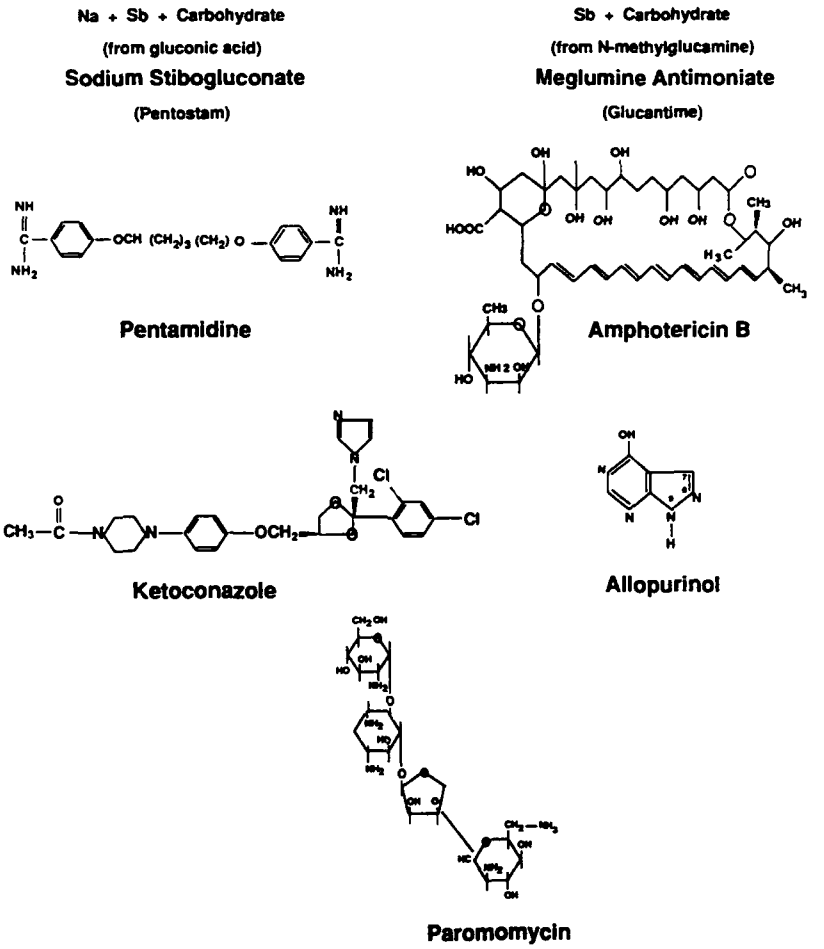

FIGURE 1. Antileishmanial agents. The structures of pentostam and glucantime are unknown. The structures of the other antileishmanial agents (pentamidine, amphotericin B, ketoconazole, allopurinol, and paromomycin) are shown.

compared to shorter courses (20 d of therapy resulted in 81% cure).[34] Mucosal leishmaniasis is the most Sb-resistant of the common forms of disease. Twenty-eight days of treatment will cure about 75% of patients with disease limited to the nose, but will fail in most patients (90% in one report) with disease that extends to the pharynx and larynx.[2,3] The above Sb regimens will result in hepatocellular enzyme elevation in about 30% of cases, diminution of T-wave height on EKG in about 30% of cases, sometimes severe arthralgias/myalgias in at least 50% of cases, and other side effects such as thrombocytopenia and pancreatitis.

TABLE 1
Clinical Leishmaniasis: Syndromes, Endemic Regions of the World, and Primary and Secondary Treatment Regimens

Syndrome	Region	Species of Leishmania	Primary treatment regimen[a]	Secondary treatment regimen[b]
Cutaneous	Old World	*L. tropica*	SB: 20 MKD for ?20 d	Pentamidine: 2 MKD QOD for 7 injections
		L. major	SB: 20 MKD for ?20 d	
	New World	*L. braziliensis*	SB: 20 MKD for 20 d	Pentamidine: 2 MKD QOD for 7 injections
		L. panamensis	SB: 20 MKD for 20 d	Ketoconazole: 600 mg/d for 28 d
		L. mexicana	SB: 20 MKD for 20 d	Ketoconazole: 600 mg/d for 28 d
Mucosal	New World	*L. braziliensis*	SB: 20 MKD for 28 d	Amphotericin B: 1 mg/kg QOD for 40 d
		L. panamensis	SB: 20 MKD for 28 d	
Visceral	India	*L. donovani*	SB: 20 MKD for 40 d	[Lipid complexed amphotericin b for visceral disease in all regions]
	Brazil	*L. chagasi*	SB: 20 MKD for 20–28 d	
	Africa	*L. donovani*	SB: 20 MKD for 28 d	Paromomycin: 15 MKD for 19 d

[a] Primary treatment with pentavalent antimony (SB) is derived from Herwaldt, B. L. and Berman, J. D., *Am. J. Trop. Med. Hyg.*, 1992, in press. With permission.

[b] Secondary treatment regimens are the author's personal views as to what might be appropriate therapy. Lipid-complexed amphotericin is in brackets because the formulations are presently in trial.

TABLE 2
Mechanism of Action of Antileishmanial Agents

Agent	Mechanism
Pentavalent antimony	Inhibition of bioenergetics
Pentamidine	[Unknown]
Amphotericin B	Intercalation into membrane sterols
Ketoconazole	Inhibition of synthesis of membrane sterols
Allopurinol	Precursor to formation of purine antimetabolite
Paromomycin	[? Inhibition of ribosomal protein synthesis]
Lipid-associated Amphotericin B	Concentration of particle and drug by *Leishmania*-containing macrophage
Interferon-gamma	Activation of *Leishmania*-containing macrophage

Pentavalent antimonials are specifically active against *Leishmania*. Perhaps because of the modest interest of qualified investigators in this agent of use only against a protozoal disease, the biochemical mechanisms of pentavalent antimony against *Leishmania* have been only slightly investigated. In isolated amastigotes, Pentostam inhibited the production of ATP by 44%, and also inhibited glycolysis by 69% and fatty acid oxidation by 67% without

inhibiting the hexose monophosphate shunt and the citric acid cycle.[35,36] No specifically inhibitable enzymes in the glycolytic pathway or in the pathway of β-oxidation have been identified. Sb is a heavy metal which could bind to SH groups of any enzyme. Another explanation for the activity of Pentostam against such disparate metabolic pathways is that Sb interferes with the general structure and function of the glycosome, the *Leishmania*-specific organelle that contains much of the bioenergetic machinery of the parasite. The intramacrophage localization of amastigotes contributes to the activity of Sb to some extent; Pentostam is concentrated by a factor of 9 by (tumor) macrophages *in vitro*.[37]

C. OTHER INJECTIBLE ANTILEISHMANIAL CHEMOTHERAPEUTIC AGENTS (PENTAMIDINE AND AMPHOTERICIN B)

Pentamidine and amphotericin B are used as secondary agents in antimonial treatment failures. The relatively slight use of these agents, due to their known and feared toxicity, makes it difficult to determine optimum treatment regimens. There is some data on the treatment of American cutaneous leishmaniasis with pentamidine. A low daily dose of 2 mg/kg was chosen to decrease toxicity, and this regimen administered every other day for 7 injections cured 82% of Guyanese patients.[38] Pentamidine disrupts the DNA of the kinetoplast-mitochondrion,[39] but the function of kinetoplastic DNA is unknown as is whether this morphologic change leads to a leishmaniacidal biochemical event.

Amphotericin B is an excellent antileishmanial agent whose use is limited by the toxic reactions of local pain, systemic fever, anemia, renal dysfunction, and hypokalemia. Amphotericin B is the only agent capable of curing mucosal patients who have failed Sb therapy. *Leishmania* and fungi both contain a 24-substituted sterol (episterol or ergosterol) in their membranes, in contrast to mammalian cells which contain the unsubstituted sterol cholesterol.[40,41] Amphotericin B preferentially interacts with 24-substituted sterols, and this preference accounts for the greater toxicity of amphotericin B toward fungi and *Leishmania* compared to mammalian cells.

D. ORALLY ADMINISTRABLE AGENTS (KETOCONAZOLE AND ALLOPURINOL)

Ketoconazole was designed to replace amphotericin B as an antifungal agent. In biosynthesis of membrane sterols, squalene is cyclized to lanosterol, and lanosterol is demethylated to form cholesterol in the case of mammalian cells, and ergosterol or the episterols in the case of fungi and *Leishmania*. Ketoconazole and other triazoles/azoles inhibit fungal lanosterol demethylation to a greater extent than mammalian lanosterol demethylation. Terbinafine, a preferential inhibitor of fungal squalene epoxidase, has also been developed as an antifungal agent. Thus, the pathway by which fungi synthesize sterols

differs from that in mammalian cells not only in the final product which is 24-substituted in fungi, but also in the substrate specificity of enzymes that perform identical reactions (squalene epoxidation and lanosterol demethylation) in fungi compared to mammalian cells.

Of the several inhibitors of fungal sterol biosynthesis, ketoconazole was the most active *in vitro*, and ketoconazole has some clinical utility. In a trial against Panamanian cutaneous leishmaniasis due to *L. panamensis*, ketoconazole cured 16 of 21 patients (73%).[42] The comparison group was treated with the low daily dose of Pentostam in use at the time (13 mg Sb/kg/d for 20 d), and only 68% were cured. However, 100% of patients with *L. panamensis* cutaneous disease can be cured with the full dose of 20 mg Sb/kg/d for 20 d.[43] Ketoconazole also cured 8 (89%) of 9 patients with *L. mexicana* cutaneous disease in Guatemala, but 7 (30%) of 23 patients with *L. braziliensis* disease in the same study.[44] Ketoconazole only cured 2 of 12 patients in French Guyana presumably with *L. guyanensis* disease.[45] In brief, ketoconazole cures most patients with *L. mexicana* and *L. panamensis* cutaneous disease from Central America. Although the spectrum of disease for which ketoconazole can be recommended would, therefore, appear to be modest, in reality ketoconazole does have a place in the antileishmanial armamentarium. Ketoconazole is the only orally administrable agent with documented activity against *Leishmania* in a controlled study. There are many clinical situations in which an alternative to parenteral therapy is highly advantageous — treatment of children, presumptive treatment of lesions from which *Leishmania* cannot be isolated, treatment of lesions that appear to be slowly self-curing, treatment of cutaneous disease from regions such as Mexico and the Old World where it is predicted that disease will self-cure within six months — and ketoconazole is being used by practitioners in these situations.

The use of allopurinol and its nucleoside is a biochemically rational approach to antileishmanial chemotherapy. *Leishmania* have a purine biosynthetic pathway with broad substrate specificity, such that the hypoxanthine and inosine analogs, allopurinol and allopurinol ribonucleoside, are phosphoribosylated to the analog of inosine monophosphate. In mammalian cells, allopurinol is poorly phosphoribosylated and primarily serves as a substrate and inhibitor of hypoxanthine catabolism to oxypurinol. In addition, the allopurinol analog of inosine monophosphate can be deaminated to the analog of AMP and then phosphorylated to the analog of ATP. Although it is unknown which of the purine analogs inhibit *Leishmania* viability, allopurinol and allopurinol ribonucleoside do have modest antileishmanial activity *in vitro*.

One problem with agents of modest activity is that demonstration of clinical efficacy requires a more carefully designed clinical trial than that required for an agent with more easily demonstrated efficacy. We have previously reviewed uncontrolled trials showing that allopurinol aided the activity of Sb in Sb-resistant cases of visceral leishmaniasis and in visceral leishmaniasis in HIV-infected persons.[46] Five patients who failed Pentostam were

retreated with the same dose of Pentostam plus allopurinol (20 mg/kg/d divided tid) for 2 to 9 weeks, and all patients healed without relapse.[47] Two cases of kala-azar in HIV-infected patients have been administered allopurinol.[48] In the first case, a patient who repeatedly parasitologically relapsed after several courses of Glucantime was treated with 20 mg/kg of allopurinol apparently divided tid for three months, at which point his bone marrow cultures were negative. In the second case, a patient who relapsed after Glucantime and after pentamidine therapy and then could not be parasitologically healed with Glucantime, was administered allopurinol for ~4 months, which resulted in negative bone marrow cultures. However, controlled trials of allopurinol have not been reported, and the clinical potential of this agent can not presently be determined.

E. TOPICAL AGENTS (PAROMOMYCIN)

Paromomycin is an aminoglycoside that is virtually identical chemically to neomycin. Both compounds consist of 2-deoxystreptamine attached to three aminosugars, but paromomycin has a (CH_2OH) substituent on one of the aminosugars, whereas neomycin has a (CH_2NH_2) substituent. In spite of this structural similarity, of the aminoglycosides only paromomycin has antiprotozoal activity, and the antiprotozoal activity is broad. Paromomycin is recommended for giardiasis and for amebiasis, and is a prime candidate for the treatment of cryptosporoidial disease in HIV-infected patients. Paromomycin is also an excellent antileishmanial agent *in vitro* with an ED_{50} comparable to that of Sb,[49] is as active as Sb against visceral disease in Kenya,[50] and is being tried as the active component of a topical preparation. It is thought that cutaneous leishmaniasis due to *L. major* in the Old World and *L. mexicana* in the New World does not involve lympathic or hematogenous dissemination from the cutaneous ulcer. In this case, a topical preparation might provide a drug to all infected tissue and might obviate the need for parenteral therapy. An initial study in which *L. major* cutaneous disease was treated with 10 d of topical paromomycin showed that the lesions healed more rapidly (by 20 d after the end of therapy) than did untreated lesions (60 d).[51] Paromomycin is now, in general, evaluated as an injectible agent against visceral, mucosal, and cutaneous disease, and as a topical agent against cutaneous disease.

F. LIPOSOMES

The obligate intramacrophage localization of *Leishmania* makes liposomal therapy attractive. Liposomes are lipid particles that are customarily removed from the circulation via macrophage phagocytosis. If the liposomes contain drugs, then the drugs will be delivered in huge quantities to the macrophages in which amastigotes reside. In addition, if the organs to which the drug is toxic do not efficiently phagocytize lipid particles, the toxicity of liposome-encapsulated drugs should be diminished. Therefore, the therapeutic index of antileishmanial agents encapsulated into liposomes should be increased both by an increase in activity of the drug and by a decrease in its toxicity.

Encapsulation of the primary agent for leishmaniasis, pentavalent antimony, was the first major attempt at liposomal therapy. The signal publication by Alving et al. showed that both liposomal Pentostam and liposomal Glucantime were 350 to 900 times more active than free Pentostam or Glucantime in the hamster model of visceral leishmaniasis.[52] In concordance with the increased activity of liposomal Sb, the concentration of Sb in rodent liver 1 to 4 d after administration of a liposomal formulation of Sb, was about 10 times the concentration when free Sb was administered (reviewed in Reference 53). The liposome used in the early work by Alving et al. was composed of dicetyl phosphate, cholesterol, and dipalmitoylphosphatidylcholine. The preparation was constituted by adding the drug to lipid solubilized in chloroform, and by evaporating the chloroform. This liposomal formulation has not been the subject of clinical investigation because of concerns about toxicity; dicetyl phosphate is not a normal bodily constituent and it might be toxic either directly or via immunological reaction as the evaporation process probably does not remove all of the chloroform.

The second major attempt to develop a clinical antileishmanial liposome derives from the desire of mycologists to provide a less toxic alternative to amphotericin B for disseminated mycoses. Lopez-Berestein formulated a liposome composed of dimyristoylphosphatidylcholine and dimyristolyphosphosphatidylglycerol (7:3) containing amphotericin B (10% by weight) (reviewed in Reference 54). It was hoped that this material would not be removed from the circulation by the kidneys, but would be bound both to free fungi and to fungi within host cells, and in this way, the toxicity of amphotericin B would be decreased and the activity increased. In fact, in early clinical experiments patients could tolerate up to 5 mg Amb (in the form of liposome)/kg/d, whereas the normal maximum dose is 1 mg Amb (free)/kg/d, and patients previously unresponsive to free amphotericin B therapy were cured (reviewed in Reference 54). Lopez-Berestein's preparation was made for individual patients on a small scale. The attractiveness of the liposomal-amphotericin B concept for the huge market for antimycotic chemotherapy has led to the development of three formulations of lipid-associated amphotericin B that are presently in clinical trial. Amphotericin B lipid complex (ABLC), made by Bristol-Myers Squibb, utilizes the same lipids as originally proposed by Lopez-Berestein. However, as a result of the large-scale manufacturing process, the material now contains 33% amphotericin B by weight, and is ribbon-like rather than circular.[55] Ambisome, made by Vestar, consists of approximately 12% amphotericin B encapsulated within phosphatidylcholine, distearoylphosphatidylglycerol, and cholesterol. This material appears to be a classic liposome on the basis of small size.[56] Amphotericin B colloidal dispersion ("amphocil"), made by Liposome Technology, consists of amphotericin B complexed with cholesteryl sulfate into a large disk of 100 nm in diameter and 4 nm in thickness.

All three liposomal preparations of amphotericin B are in clinical trial as antimycotic agents, and therefore, are available for trial as antileishmanial

agents. A rational approach toward choosing the best preparation would involve determining which preparation is least concentrated in the kidney and most concentrated in the liver, spleen, and bone marrow (for visceral leishmaniasis) or in the skin and mucous membranes (for cutaneous and mucosal disease). In reality, such a comparison is difficult to perform because the pharmacokinetics of the compounds have been determined in separate experiments under noncomparative conditions. A further complicating factor is that free amphotericin B is itself a particle. Amphotericin B is insoluble in aqueous solution; the clinical preparation that is termed "amphotericin B" is, in reality, a deoxycholate micellar suspension of the substance amphotericin B. If the deoxycholate is not metabolized in the serum (this point is unclear), then "free amphotericin B" is, in fact, a lipid-associated drug and any improvement by the three new preparations over free amphotericin B is not due to simply having drug associated with lipid, but to more subtle differences between the serum pharmacokinetics and targeting of new lipid-associated particles vs. the old lipid-associated particle.

Nevertheless, compared to amphotericin B deoxycholate, all three new formulations of lipidassociated amphotericin B do seem to be more efficiently removed from the circulation, to be more concentrated in liver, spleen, and bone marrow, and to be less concentrated in kidney. Serum pharmacokinetic data are available for ABLC and Amphocil. When humans were administered either ABLC or Amb deoxycholate at an Amb concentration of 0.25 mg/kg, the maximum serum concentration and serum half-life for ABLC were 214 ng/ml and 27 h, whereas the maximum serum concentration and serum halflife for Amb deoxycholate was 984 ng/ml and 50 h. ABLC had a volume of distribution of 2.6 l/kg and Amb deoxycholate was distributed to 0.74 l/kg.[55] In the dog, mean Amb plasma levels after 0.6 mg Amb in the form of amphocil were approximately one fifth the plasma levels after 0.6 mg Amb in the form of Amb deoxycholate.[57]

Published data on the distribution of Ambisome and Amphocil vs. amphotericin B deoxycholate in dog and rat, respectively, is shown in Table 3. This table indicates that for both new formulations of amphotericin B, the site of increased tissue deposition is primarily the liver, and the tissue which is specifically spared is the kidney. Thus, dogs and rats can be administered 4 to 5 times more Amb in the form of Amphocil or Ambisome than in the form of amphotericin B deoxycholate before comparable kidney concentrations are reached, and the higher levels of administered drug result in a 5 to 10 times higher level of Amb in the liver and spleen.

In preclinical activity studies, each of the three new formulations of Amb was shown to be more active than amphotericin B deoxycholate. ABLC was 4 times more active than Amb in the lightly infected hamster model of visceral leishmaniasis and as active as Amb in the heavily infected hamster model;[58] Amphocil was 15 times more active than Amb in the lightly infected hamster and 4 times more active in the heavily infected hamster (Hanson et al., unpublished data); Ambisome was approximately 4 times more active in the mouse model.[59]

TABLE 3
Biodistribution of Lipid-Associated Amphotericin B Formulations Compared to Amphotericin B Deoxycholate

Drug	AmB			AmB		
Dose (mg/kg)	deoxycholate 0.6	Amphocil 0.6	Amphocil 2.5	deoxycholate 1.0	Ambisome 1.0	Ambisome 5.0
Animal	Dog	Dog	Dog	Rat	Rat	Rat
Liver	116	197	626	32	84	347
Spleen	80	87	419	37	53	396
Bone marrow	2.7	7.5	na	na	na	na
Kidney	82	12	57	6.4	1	7.1

Note: Data shown is concentration of AmB in tissues (μg Amb/g tissue). Dog data is from Reference 57. Rat data is from Reference 56.

The only report of clinical activity of lipid associated Amb is a case report of one patient who failed to be cured with multiple standard antileishmanial agents and was then cured with 1.0 mg Amb (in the form of Ambisome)/kg/d for 21 d.[60] Although this was a single case, the success of this case, combined with the attractiveness of administering an excellent antileishmanial agent whose use was previously limited by toxicity, in a less toxic form has resulted in an World Health Organization initiative to conduct clinical trials of all three agents in the several endemic regions of visceral leishmaniasis.

G. IMMUNE STIMULATORS (IFN-γ)

The obligate intramacrophage localization of *Leishmania*, and the ability of INF-γ to cure infected macrophages in models, has led to clinical trials of INF-γ. Harms et al. injected INF-γ directly into cutaneous lesions. Although 25 μg INF-γ injected once weekly for 5 consecutive weeks partially healed 13 of 37 lesions, 1.3 ml of Sb injected on the same schedule completely healed 29 of 38 lesions and partially healed the other 9 lesions.[61] Badaro et al.[62] reported the effect of the combination of Pentostam and INF-γ vs. visceral disease that was previously resistant to Pentostam alone and or that was previously untreated. Six of eight cases of previously antimony-resistant disease was cured with the administration of antimony and INF-γ. Eight of nine naive cases were also cured with the combined regimen[62] This exciting but preliminary work suffers from lack of controls; the rate of cure of previously resistant disease to a second treatment with Pentostam alone is unknown and the cure rate of naive cases in Brazil to Pentostam alone is not well documented.

The combination of INF-γ with standard agents is presently in clinical trial against cutaneous, mucosal, and visceral leishmaniasis. Although the approach of combining immunomodulator therapy with chemotherapy is theoretically attractive, there are also underlying difficulties with this approach. Systemic administration of immunomodulators will result in systemic toxicity by these pluropotential agents. Addition of another injectible agent to a parenteral chemotherapeutic regimen is undesirable and these agents are newly developed and, therefore, expensive. If immunomodulators provide merely a modest improvement upon chemotherapy alone, the immunomodulators will have little practical use.

V. SUMMARY AND CONCLUSIONS

In mammals, *Leishmania* are only found within the phagolysosomes of monocytes/macrophages. The obligate intramacrophage localization of Leishmania has enlarged the field of antileishmanial agents from compounds active *in vitro* to include agents that are active because they are concentrated by macrophages or because they immunologically stimulate macrophages. The classical injectible agents are pentavalent antimony in the form of Pentostam or Glucantime, and the secondary agents pentamidine and amphotericin B. These agents are active *in vitro* as well as clinically. The mechanism of action for pentavalent antimony is inhibition of parasite bioenergetics; the mechanism of action of amphotericin B is, as for fungi, intercalation with microorganism 24-substituted membrane sterols. The oral agents ketoconazole and allopurinol are moderately active. Their mechanisms are inhibition of sterol biosynthesis (ketoconazole) and being the precursor for abnormal purines (allopurinol). The aminoglycoside paromomycin is a recently recognized antileishmanial agent whose activity against this and other protozoa is as yet unexplained. New modalities of treatment that specifically take advantage of the intramacrophage localization of the organisms are chemotherapy with lipid-associated amphotericin B and immunotherapy with INF-γ. The fact that lipid-associated amphotericin B is removed from the circulation and kept from the kidney by the macrophages in which visceralizing *Leishmania* reside, suggests that lipid-associated amphotericin B may soon replace antimonial therapy for visceral leishmaniasis. Stimulation of macrophages to kill their intracellular parasite with INF-γ is a theoretically attractive treatment option that, however, is likely to be limited by the cost, requirement for parenteral injection, toxicity, and as yet undocumented efficacy of this agent. Nevertheless, the initial success of lipid-associated amphotericin B and the interest in immunotherapy of leishmaniasis suggests a strong future for agents specifically targeted to intramacrophage microorganisms.

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